US-PAT-NO: 6570061

DOCUMENT-IDENTIFIER: US 6570061 B1

TITLE: Targeted replacement of an immunoglobulin gene without endogenous and selectable residual sequences in mice

DATE-ISSUED: May 27, 2003

INVENTOR-INFORMATION:

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US-CL-CURRENT: **800/18**, 435/320.1 , 435/325 , 435/326 , 435/352 , 435/354 , 435/455 , 435/462 , 435/463 , 435/69.1 , 435/69.2 , 435/69.3 , 435/69.4 , 435/69.6 , 435/71.1 , 530/387.1 , 800/22 , 800/24 , 800/4 , 800/5

ABSTRACT:

Transgenic mice that produce high levels of humanized antibodies are described. Targeted gene replacement exchanges constant regions of the mouse immunoglobulin **heavy and light** chain genes with human genes, either through conventional gene targeting, or by use of the bacteriophage-derived Cre-loxP recombination system. The transgenic animals undergo antibody affinity maturation, and a class switch from the native immunoglobulin to the humanized form.

31 Claims, 12 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 8

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Abstract Text - ABTX (1):

Transgenic mice that produce high levels of humanized antibodies are described. Targeted gene replacement exchanges constant regions of the mouse immunoglobulin **heavy and light** chain genes with human genes, either through conventional gene targeting, or by use of the bacteriophage-derived Cre-loxP recombination system. The transgenic animals undergo antibody affinity maturation, and a class switch from the native immunoglobulin to the humanized form.

Brief Summary Text - BSTX (6):

Various technologies have been developed to overcome problems related to the production of human monoclonal antibodies, one strategy is the generation of chimeric antibodies in which the rodent constant (C) regions of both **heavy** (H) and **light** (L) chains, with or without the framework of the variable region, are replaced by the equivalent domains or sequences of human immunoglobulin. Another strategy attempts to mimic the immune response in vitro, through bacteriophage expression of human variable region genes isolated from human B cell populations, followed by selection for rare, high affinity antibodies through antigen binding. A major drawback to these and similar approaches is the cumbersome work required to generate each specific mAb of appropriate biological function.

Brief Summary Text - BSTX (14):

The genes encoding human and mouse immunoglobulins have been extensively characterized. Berman et al. (1988) EMBO J. 7:727-738 describe the human Ig VH locus. Sakano et al. (1981) Nature 290:562-565 describe a diversity segment of the immunoglobulin **heavy** chain genes. Blankenstein and Kruwinkel (1987) Eur. J. Immunol. 17:1351-1357 describe the mouse variable **heavy** chain region.

Drawing Description Text - DRTX (2):

FIGS. 1A to 1C are schematics of the human and mouse **immunoglobulin** kappa region **loci**. FIG. 1A shows the germline mouse C.sub..kappa. locus. FIG. 1B shows the subject targeting vector. FIG. 1C shows the mouse locus after the recombination event in which mC.sub..kappa. is replaced by HC.sub..kappa.. The cleavage sites for restriction enzymes (B, BamHI; E, Eco RI; H, Hpa I; M, Mst II; Bg, Bgl II; K, Kpn I) are indicated. The DNA probes used for the Southern blot analyses are shown, as well as the fragments obtained. FIG. 1C shows the primers, designated by arrows, used for amplification.

Detailed Description Text - DETX (3):

The subject invention provides for the production of polygonal humanized anti-serum or humanized monoclonal antibodies. The humanized antibodies have a human constant region and a host variable region. The humanized antibodies are produced at a level comparable to native antibodies. Humanized **light** chains will usually be present in serum at concentrations of at least about 500 .mu.g/ml, more usually at least about 1 mg/ml. The serum concentration of humanized **heavy** chains is dependent on class switching and will usually be present at concentrations of at least about 50 .mu.g/ml, more usually at least about 100 .mu.g/ml. The genes encoding the humanized antibodies are able to undergo somatic hypermutation, thereby allowing for B cell selection and affinity maturation.

Detailed Description Text - DETX (4):

The subject transgenic animals have a native immunoglobulin (Ig) constant region gene functionally replaced with a human constant region gene, that is, the human constant region segment replaces the native gene segment in the

genetic recombination and expression events associated with an antibody response. The native gene may be deleted or inactivated. The constant region gene is herein defined as the constant region exons, and optionally including introns, encoding, the secreted portion of a mature immunoglobulin chain. In a preferred embodiment, the host transmembrane and cytoplasmic portion will be retained. An intact switch region, either human or from the native gene, will be present at the **heavy** chain locus.

Detailed Description Text - DETX (5):

For most applications, it is desirable to have the genes for both the Ig **heavy and light** chain constant regions replaced with human genes. Either of the human **light** chain constant region genes, i.e. C.kappa. and C.lambda., may be used to replace a host **light** chain constant region. At the host **heavy** chain locus, at least one of the isotypes will be functionally replaced, e.g. C.mu., C.delta., C.gamma., C.alpha. or C.epsilon.. The transgenic human gene may be the counterpart to the native gene, e.g. C.gamma.1.fwdarw.C.gamma.1, or may be a different isotype. Preferably, the replaced host region will be other than C.mu.. Of particular interest are the .alpha. and .gamma. constant regions, which may be interchanged, e.g. C.gamma.1.fwdarw.C.alpha.; C.gamma.2.fwdarw.C.alpha.; C.gamma.3.fwdarw.C.alpha.; C.gamma.4.fwdarw.C.alpha.; C.alpha..fwdarw.C.gamma.1, etc., C.gamma.1.fwdarw.C.epsilon., etc.; C.alpha..fwdarw.C.epsilon., and the like.

Detailed Description Text - DETX (7):

Methods for producing transgenic animals are known in the art. A host embryonic cell, generally an embryonic stem cell line, is transfected with the recombination vector. Where the exogenous gene is Ig **heavy** chain, the host coding region for the exons CH1, CH2, hinge, CH3 and CH4 will be inactivated by a lesion that results in the loss of transcription. Preferably, the **heavy** chain cytoplasmic and transmembrane domains of the constant region will continue to be expressed. Where the exogenous gene is an Ig **light** chain, at least one of the host Ig **light** chain constant regions, e.g. IgC.kappa. or IgC.lambda., will be similarly inactivated. Such a lesion may take the form of a deletion in the target gene, an insertion of a foreign gene, or a replacement, where a deletion is made in the endogenous gene and is replaced with exogenous sequences. In a preferred embodiment, the vector will include loxP sites, allowing for the deletion of the host coding region through the action of Cre recombinase.

Detailed Description Text - DETX (28):

The direct proof that the HC.sub..kappa. gene functionally replaced the mC.sub..kappa. gene is shown by an analysis of antibody-producing cells (B-lymphocytes) in the mouse. Resting B-lymphocytes carry antibodies on their surface that they express after gene rearrangement. These antibodies can be detected by labeled antibodies specific for **light** chains. FIG. 2 depicts the results of an experiment in which antibodies specific either for the kappa chain of the mouse or for the kappa chain of humans. The cells were stained with a phycoerythrin (PE) conjugated antibody that recognizes all B-cells (anti CD45R(B220)). At the same time, the cells were stained with a fluorescein

conjugated antibody specific for the constant region of the kappa-**light** chain. The data shows that B-lymphocytes in the transgenic mouse express antibodies with the human kappa chain and that the kappa chain of the mouse is no longer used.

Detailed Description Text - DETX (42):

In the transgenic mouse strain, substantial numbers of B-cells which express humanized .kappa. chains on the cell surface are generated. In the blood of 8-week-old transgenics, the levels of antibodies bearing humanized **light** chains were approximately 2 mg/ml, compared to 3.5 mg/ml .kappa. chain bearing antibodies in control mice of the same age.

Detailed Description Text - DETX (44):

The distribution of antibody isotypes was similar in mutant and wild-type animals. Although in the transgenics about 10% of the antibodies carry .gamma..sub.1 chains, a large fraction of the IgG antibodies are associated with humanized .kappa. chains, because IgG represents the major isotype in the serum. The presence of serum IgG antibodies which carry the humanized **light** chains indicates that B cells expressing the latter can be triggered by environmental antigens to contribute to antibody responses.

Detailed Description Text - DETX (51):

On day 14 of the phOX-CSA response, splenocytes from a C.kappa.R mutant mouse were isolated and stained with FITC-conjugated RA3-2B6 and PE-conjugated PNA, followed by sorting for the PNA.sup.hi B cell population. The purity of the sorted cells was 91%. cDNA sequences of V.kappa..sub.OX1 -J.kappa..sub.5 were obtained and compared to the germline gene by the following method. Total cellular RNA was prepared from 1.8.times.10.sup.5 PNA.sup.hi splenic B cells (as described in Gu et al. (1990) EMBO J. 9:2133). cDNA was synthesized using the Super Script Reverse Transcriptase kit (BRL). V.kappa..sub.OX1 -J.kappa.5 joints were then amplified with synthesis primers carrying the cloning sites of BAMHI and HindII. The primers were as follows: V.kappa..sub.OX1 leader specific primer (SEQ ID NO:16) TGCGGATCCTCAGTCATAATATCCAG and J.kappa..sub.5 primer (SEQ ID NO:17) CGGAATTCTTTCAGCTCCAGCTTGG. PCR was performed for 35 cycles. Each cycle consisted of 1 min. at 94.degree., 1 min. at 60.degree., and 1 min. at 74.degree.. The amplified **light** chain fragments were cloned into the PTZ19R vector (Pharmacia, Uppsala).

Detailed Description Text - DETX (52):

The data is shown in Table 2. The data revealed that 66% of the sequences had mutations in the V.kappa..sub.OX1 -J.kappa..sub.5 region of the chimeric k chain. Several sequences carried the key mutations in codons 34 and 37, known to increase the affinity of phOX-binding antibodies by approximately 10-fold. The frequency of mutations (2 mutations/sequence) in the chimeric **light** chains is similar to previously published observations.

Detailed Description Text - DETX (58):

Serum levels of antibodies of mutant and wild-type animals were determined by ELISA. Each symbol represents a value obtained from an individual mouse. FIG. 5A: Serum concentrations of Ig isotypes in 7-week-old mutant mice. Sera from 5-week-old wild-type 129 mice served as control. For the determination of humanized IgG1, plastic plates were coated with g)oat anti-human IgG antibodies (Jackson Immuno Research) and developed with mouse mAb anti-human IgG1 (clone 8c/6-39, The Binding Site, Birmingham, UK). ELISA was performed as described for all the other isotypes. The concentrations were calculated with mAbs of the respective isotypes as standards. The concentrations of **light** chain isotypes were determined as well, and the ratio of .kappa.- to .gamma.1-bearing antibodies in the mutant animals was found to be around 4.5, indicating that the majority of the **heavy** chains pair with the chimeric .kappa. chains.

Detailed Description Text - DETX (66):

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in **light** of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

Claims Text - CLTX (3):

3. The method of claim 1 wherein said immunoglobulin constant region gene segment is a **heavy** chain gene.

Claims Text - CLTX (4):

4. The method of claim 1 wherein said immunoglobulin constant region gene segment is a **light** chain gene.

Claims Text - CLTX (15):

15. The transgenic mouse of claim 13 wherein said immunoglobulin constant region gene segment is a **light** chain gene.

Claims Text - CLTX (16):

16. The transgenic mouse of claim 13 wherein said immunoglobulin constant region gene segment is a **heavy** chain gene.

Claims Text - CLTX (21):

21. The transgenic mouse according to claim 13, wherein said mouse comprises a human C.kappa. gene segment inserted at the locus for an endogenous mouse immunoglobulin **light** chain constant region gene segment on both chromosomes and a human C.gamma.1 constant region gene segment, wherein said antibodies produced comprise domains from human Ck, and human C.gamma.1.

Claims Text - CLTX (23):

23. The method of claim 22 wherein said immunoglobulin constant region gene

segment is a **light** chain gene.

Claims Text - CLTX (24):
24. The method of claim 22 wherein said immunoglobulin constant region gene segment is a **heavy** chain gene.

Claims Text - CLTX (29):
29. The method of claim 22, wherein said mouse comprises a human Ck gene segment inserted at the locus for an endogenous mouse immunoglobulin **light** chain constant region gene segment on both chromosomes and a human C.gamma.1 constant region gene segment, wherein said antibodies produced comprise domains from human Ck, and human C.gamma.1.

Claims Text - CLTX (31):
31. A method of producing a transgenic mouse comprising a human immunoglobulin **light** chain gene segment and a human immunoglobulin **heavy** chain gene segment comprising mating a first transgenic mouse having a genome comprising a targeted, functional replacement of a mouse immunoglobulin constant region gene segment with a human immunoglobulin constant region gene segment, wherein in said first transgenic mouse, said human immunoglobulin constant region gene segment is from the C.gamma.1 gene, with a second transgenic mouse having a genome comprising a targeted, functional replacement of a mouse immunoglobulin constant region gene segment with a human immunoglobulin constant region gene segment, wherein in said second transgenic mouse said human immunoglobulin constant region gene segment is from the Ck gene and selecting progeny that comprises a gene segment from the C.gamma.1 gene and a gene segment from the Ck gene.

Related Application Filing Date - RLFD (1):
19930824

Current US Original Classification - CCOR (1):
800/18